

### **REMARKS**

Claims 1-23 are all of the claims pending in the application.

Claims 1-3, 6, 7 and 9-12 are amended herein, and new claims 24-30 are added. No new matter within the meaning of 37 C.F.R. § 1.121(f) is added. Accordingly, entry of these amendments and new claims is requested.

New claims 24-30 are added to better define that which the Applicants regard as their invention and are supported, for example, at pp.5-6 (claims 24-26); pp. 10-12 (claim 27); pp. 9-11 (claim 28 and 29); and pp. 14-16 (claim 30) of the specification.

#### **Rejections of claims under 35 U.S.C. § 112, ¶ 2**

(a) Claims 4-6, 13, 14, 16, 17 and 20 are rejected under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite for containing unconventional claim language and superfluous words.

Applicants assert that the rejection of claims 4-5, 13, 14, 16, 17 and 20 is rendered moot by their cancellation. Applicants further assert that new claims 24-30 do not contain the unconventional claim language and superfluous words objected to by the Examiner.

With respect to claims 6, Applicants assert that the amendments to claim 6 herein render this rejection moot. Specifically, the claim is amended to delete the objected-to phrase “characterized in that.”

Accordingly, Applicants request that this rejection of claims 4-6, 13, 14, 16, 17 and 20 under 35 U.S.C. § 112, second paragraph, be withdrawn.

(b) Claims 1-21 are rejected under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite because phrases such as “biotin-introduced product” are allegedly unclear as to whether they refer to a biotinylated substance or just to a biotin.

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Applicants assert that the withdrawal of claims 4, 5 and 13-21 herein renders the rejection moot as to those claims.

With respect to rejected claims 1-3 and 6-12, claims 1-3, 6 and 9-11 are amended herein to replace the objected-to phrase “biotin-introduced” with the phrase “biotinylated” to clarify that the phrase encompasses substances that are biotinylated. Applicants assert that the amended claims are not indefinite, and that they claim with reasonable clarity and precision that which Applicants regard as their invention. Support for these amendments may be found, for example, at page 7, fourth full paragraph, of the specification, and at pages 4-10 generally.

Accordingly, Applicants request that the this rejection of claims 1-21 under 35 U.S.C. § 112, second paragraph, be withdrawn.

(c) Claims 4 and 5 are rejected under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite for lacking method steps.

Applicants assert that this rejection is rendered moot by the cancellation herein of claims 4 and 5, and request that the rejection be withdrawn.

(d) Claim 6 is rejected under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite for failing to recite clear, active steps.

Claim 6 is amended herein to recite three clear steps that are recited in the active tense (i.e. providing, bringing and analyzing). Therefore, Applicants assert that the amendment to claim 6 renders the present rejection moot, and request that the rejection be withdrawn.

**Objection under 37 C.F.R. § 1.75(c)**

Claims 9 and 12 are objected to under 37 C.F.R. § 1.75(c) as being allegedly in improper form because a multiple dependent claim cannot properly depend from another multiple dependent claim.

Applicants assert that the objections to claim 9 and claim 12 are rendered moot by (1) the cancellation herein of claim 5, (2) the amendment to claim 7 to depend only from claim 6, (3) the amendment to claim 9 to depend from claims 6-8, and (4) the amendment to claim 12 to depend from claims 6-8. Thus, as amended, claim 7 is not a multiple dependent claim and therefore Applicants assert that claims 9 and 12 as amended are not improper.

Accordingly, in view of the above remarks and amendments, Applicants respectfully request examination of claims 9 and 12.

**Rejections under 35 U.S.C. § 103**

(a) Claims 1-7, 9 and 12-23 are rejected under 35 U.S.C. § 103(a) as being allegedly obvious over Haugland et al. (U.S. Patent No. 5,443,986) in view of Sano (U.S. Patent No. 6,022,951).

Applicants respectfully disagree with the Examiner's characterization of the teaching of Haugland et al. at page 3, line 9, of the Office Action. While Haugland et al. may teach a biotin-avidin-biotin complex comprising two different biotinylated products (see, Haugland et al., Table 12), Applicants assert that it does neither teach nor suggest such a complex comprising a cross-linked avidin, as stated by the Examiner. In this regard, Applicants note that the Examiner later states that Haugland et al. fails to teach a cross-linked avidin.

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With respect to rejection of claims 4, 5, and 13-23, Applicants assert that the rejection is rendered moot by their cancellation.

With respect to claims 1-3, 4-7, 9 and 12, Applicants direct their comments to independent claims 1 and 6, because if these claims are found allowable, the balance of the rejected claims dependent therefrom will also be found allowable.

Applicants traverse the rejection on the grounds that (1) Haugland et al. and Sano et al. do not together teach or suggest all the elements of the rejected claims, (2) that the combination of the references is improper because Sano et al. teaches away from their combination, and (3) that there would be a lack of motivation for one of ordinary skill to combine the references and no reasonable expectation of success in doing so in view of the state of knowledge at the time of filing the present application.

First, Applicants assert that neither Haugland et al. nor Sano et al. teaches or suggests the complex of claim 1 and 6, which comprises two biotinylated products and a crosslinked avidin sandwiched therebetween. Specifically, Haugland et al. may disclose a biotin-avidin-biotin complex comprising two biotinylated products that may be different. However, as noted by the Examiner, Haugland et al. does not teach or suggest a cross-linked avidin. Similarly, Sano et al. may disclose a crosslinked streptavidin and its complexes, but it does not teach or suggest complexes comprising a crosslinked avidin. Accordingly, Applicants assert, in view of the comments below, that neither reference teaches or suggests the complex of claim 1 and 6, which comprises two biotinylated products and a crosslinked avidin sandwiched therebetween, and

therefore the combined references do not teach or suggest all of the elements of the rejected claims.

Second, Applicants assert that the combination of Haugland et al. and Sano et al. is improper because although Sano et al. may teach the use of crosslinked streptavidin, it specifically teaches away from the use of crosslinked avidin. It is improper to combine references where the references teaches away from their combination. MPEP 2145.

Specifically, Sano et al. first teaches that streptavidin and avidin are different in many respects (col.1, ¶3), including having different molecular weights, difference electrophoretic mobilities, different glycosylation, and different amino acid composition. Sano et al. further teaches that avidin is less suitable than streptavidin for many applications because of its propensity to bind non-specifically to biological materials including cell nuclei, nucleic acids and lectins (col.1, last two lines of ¶3). Thus, Applicants assert that Sano et al. clearly teaches away from the use of avidin in favor of the use of streptavidin. In view of the fact that Sano et al. teaches away from the use of avidin, Applicants assert that the combination of Sano et al. and Haugland et al. is improper. Further, in view of the negative teaching of Sano et al. with respect to avidin, one of ordinary skill would not have been motivated to combine the crosslinking taught in Sano et al. with the avidin complexes taught in Haugland. Similarly, one of ordinary skill would not have had a reasonable expectation of success in combining the teachings of Sano et al. and Haugland et al. to produce a complex comprising a crosslinked avidin because Sano et al. teaches both the differences between streptavidin and avidin and also teaches away from the use of avidin.

Third, Applicants assert that one of ordinary skill would not have been motivated to combine the cited references, nor would have had a reasonable expectation of success in doing so, because neither of the references, nor the general level of knowledge available in the art at the time of filing, would have taught or suggested a reason for their successful combination. Specifically, the present specification discloses an attempt to use a complex comprising a biotinylated antibody, avidin, and a biotinylated enzyme that was not suitable for use as an analyzing reagent because the reactivity and stability of the complex were insufficient (see, specification pp.3-4). Applicants assert that the reason for the insufficient reactivity and stability of the uncrosslinked avidin complex were not understood prior to the date of conception of the present invention. Applicants therefore assert that for as long as the cause of the deficiency was not understood, it would not have been obvious to one of ordinary skill to apply the crosslinking disclosed in Sano et al. to the avidin of the biotin-avidin-biotin complex taught by Haugland et al. Thus, Applicants assert that it would not have been obvious to try the crosslinking taught in Sano et al. in the complex of Haugland et al. because one of ordinary skill in the art would not have expected that a complex of crosslinked avidin and two dissimilar biotinylated products would exhibit increased stability as a whole because the reason why such a complex comprising uncrosslinked avidin lacked stability was not understood.

For the foregoing reasons, Applicants request that the rejection under 35 U.S.C. § 103(a) over Haugland et al. (U.S. Patent No. 5,443,986) in view of Sano (U.S. Patent No. 6,022,951) be withdrawn.

(b) Claims 8, 10 and 11 are rejected under 35 U.S.C. § 103(a) as being allegedly obvious over Haugland et al. in view of Tatsumi et al. (U.S. Patent 5,843,746).

The Examiner's position appears to be that Haugland et al. teaches all elements of Claims 8, 10 and 11, except where the binding component is a Fab' fragment or an enzyme expressed as a biotinylated fusion-protein, and that Tatsumi et al. teaches a luciferase biotinylated fusion-protein. The Examiner asserts that one of ordinary skill in the art would readily have substituted the luciferase biotinylated fusion-protein of Tatsumi et al. for the biotinylated enzyme of the biotin-avidin-biotin complexes taught in Haugland et al.

Although the Examiner does not explicitly include Sano et al. with Haugland et al. and Tatsumi et al. in making this rejection, Applicants believe this may be the intent and proceed on that assumption. Sano et al. appears to be necessary to the rejection because it alone may teach cross-linked avidin.

Applicants respectfully traverse the rejection. Applicants assert that Tatsumi et al. does not cure any of the deficiencies noted above in relation to the rejection under 35 U.S.C. § 103(a) over Haugland et al. in view of Sano. Thus, Applicants first assert that Tatsumi et al. fails to teach or suggest the complex of claim 6, from which the rejected claims depend, in which the complex comprises two biotinylated products and a crosslinked avidin sandwiched therebetween. Second, Applicants reassert that Sano et al. specifically teaches away from the use of crosslinked avidin, and that Tatsumi et al. cannot overcome that teaching. Third, Applicants assert that Tatsumi et al. does not cure the lack of motivation to combine Haugland et al. and Sano et al., nor does it cure the lack of reasonable expectation of success in doing so.

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With respect to Claim 8, Applicants further assert that the Examiner has not made a *prima facie* case of obviousness. Specifically, none of the three cited references teaches the use of Fab' fragments. Therefore, not all the elements of the Claim 8 are taught in the combined references.

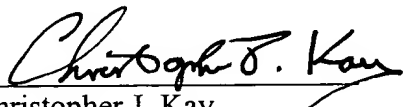
Therefore, Applicants request that the rejection of claims 8, 10 and 11 under 35 U.S.C. § 103(a) as being allegedly obvious over Haugland et al. in view of Tatsumi et al. be withdrawn.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

Applicant hereby petitions for any extension of time in addition to the petition for a two month extension of time submitted herewith which may be required to maintain the pendency of this case, and any required fee, except for the Issue Fee, for such extension is to be charged to Deposit Account No. 19-4880.

Respectfully submitted,

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**APPENDIX**  
**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**IN THE CLAIMS:**

**Claims 4, 5 and 13-23 are canceled.**

**The claims are amended as follows:**

1. (Amended) A biotin-avidin-biotin complex comprising at least two [biotin-introduced products] biotinylated substances which are the same or different, and a crosslinked avidin sandwiched therebetween.
2. (Amended) The biotin-avidin-biotin complex according to claim 1, wherein at least one of said [biotin-introduced products] biotinylated substances is a [biotin-introduced] biotinylated binding component and at least one of said [biotin-introduced products] biotinylated substances is a [biotin-introduced] biotinylated labeling substance.
3. (Amended) A process for preparing said biotin-avidin-biotin complex according to claim 1, comprising the steps of:
  - (3) treating an avidin with a crosslinking agent to prepare a crosslinked [avidin,] avidin;
  - (4) biotinylating the same or different substances to be biotinylated to prepare the same or different [biotin-introduced products,] biotinylated substances; and
  - (3) binding said crosslinked avidin and said same or different [biotin-introduced products] biotinylated substances to form said biotin-avidin-biotin complex according to claim 1.
6. (Amended) A method for analyzing a compound to be [analyzed characterized in that] analyzed, said method comprising the steps of:

[(1) a sample possibly containing said compound to be analyzed, a biotin-introduced binding component capable of binding specifically to said compound to be analyzed, a crosslinked avidin, and a biotin-introduced labeling substance are brought into contact with each other, in any sequential order,]

(1) providing a sample suspected of containing said compound to be analyzed;

(1) bringing into contact sequentially and in any order said sample, a biotinylated binding component capable of specifically binding said compound, a crosslinked avidin, and a biotinylated labeling substance, to form a complex of said compound to be analyzed, said [biotin-introduced] biotinylated binding component, said crosslinked avidin, and said [biotin-introduced] biotinylated labeling substance; and

[(2)] (3) analyzing a signal derived from said labeling substance in said complex.

7. (Amended) The analyzing method according to claim [5 or] 6, wherein said binding compound is selected from the group consisting of an antibody, an antibody fragment, an antigen, a DNA, an RNA, a receptor, a ligand to a receptor, an enzyme, a ligand to an enzyme, an enzyme analogue, a substrate for an enzyme which is an origin of an enzyme analogue, a lectin, [or] and a sugar.

9. (Amended) The analyzing method according to any one of claims [5] 6 to 8, wherein said [biotin-introduced] biotinylated labeling substance is selected from the group consisting of a [biotin-introduced] biotinylated enzyme, a [biotin-introduced] biotinylated fluorescent substance, [or] a protein bound to a [biotin-introduced] biotinylated fluorescent substance, a [biotin-introduced] biotinylated luminescent substance, [or] a protein bound to a [biotin-introduced]

biotinylated luminescent substance, [or] and a [biotin-introduced] biotinylated radioactive isotope.

10. (Amended) The analyzing method according to claim 9, wherein said [biotin-introduced] biotinylated enzyme is a [biotin-introduced] biotinylated fused-protein of an enzyme and a biotin acceptor.

11. (Amended) The analyzing method according to claim 9, wherein said [biotin-introduced] biotinylated enzyme is a [biotin-introduced] biotinylated luciferase.

12. (Amended) The analyzing method according to any one of claims [4 to 11,] 6 to 8, wherein said crosslinked avidin is selected from the group consisting of a crosslinked egg-white avidin, a crosslinked streptoavidin, [or] and a crosslinked recombinant avidin.

**Claims 24-30 are added as new claims.**

24. (New) The analyzing method according to claim 9, wherein said crosslinked avidin is selected from the group consisting of a crosslinked egg-white avidin, a crosslinked streptoavidin, and a crosslinked recombinant avidin.

25. (New) The analyzing method according to claim 10, wherein said crosslinked avidin is selected from the group consisting of a crosslinked egg-white avidin, a crosslinked streptoavidin, and a crosslinked recombinant avidin.

26. (New) The analyzing method according to claim 11, wherein said crosslinked avidin is selected from the group consisting of a crosslinked egg-white avidin, a crosslinked streptoavidin, and a crosslinked recombinant avidin.

27. (New) An analyzing reagent comprising a mixture of:

- (1) a biotinylated binding component;
- (2) a crosslinked avidin; and
- (3) a biotinylated labeling substance.

28. (New) The analyzing reagent of claim 27, wherein said binding component is selected from the group consisting of an antibody, an antibody fragment, an antigen, a DNA, an RNA, a receptor, a ligand to a receptor, an enzyme, a ligand to an enzyme, an enzyme analogue, a substrate for an enzyme which is an origin of an enzyme analogue, a lectin, and a sugar.

29. (New) The analyzing reagent of claim 28, wherein said antibody fragment is an Fab' fragment.

30. (New) A method for analyzing a compound to be analyzed, said method comprising the steps of:

- (1) providing a sample suspected of containing said compound to be analyzed;
- (2) providing a biotin-avidin-biotin complex comprising a biotinylated binding component and a biotinylated labeling substance, and a crosslinked avidin sandwiched therebetween;
- (3) bringing said sample into contact with said biotin-avidin-biotin complex to form a complex of said compound to be analyzed and said biotin-avidin-biotin complex; and
- (4) analyzing a signal derived from said labeling substance in said complex formed in step (3).